

NEW TECHNOLOGIES FOR INFECTIOUS AND TROPICAL DISEASES

Detection and monitoring of human bocavirus I infection by a new rapid antigen test

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Abstract

Clinically relevant diagnosis of human bocavirus I (HBoVI) is challenging, as the virus is frequently detected in asymptomatic patients, and cofindings with other respiratory viruses are common. The clinical value of current diagnostic methods, such as PCR, is therefore low, and alternative diagnostic strategies are needed. We describe for the first time the use of an antigen detection assay for the rapid identification of HBoVI in a paediatric patient with respiratory tract infection symptoms. We estimate the duration of active HBoVI infection to be 6 days.

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Introduction

Human bocaviruses (HBoVs) are small single-stranded DNA viruses which belong to the family *Parvoviridae* (subfamily *Parvovirinae*). Currently four HBoV species have been identified. Human bocavirus I (HBoVI) was described for the first time in 2005 in children with respiratory tract infection (RTI) [1]. Three other HBoVs, designated HBoV2, HBoV3 and HBoV4, have mainly been detected in faeces, but a causal relation with disease such as gastroenteritis is still unclear [2]. Prevalence studies using nucleic acid amplification methods show that HBoVI can be detected in 1.6 to 21.5% of children with symptoms of RTI, particularly during winter and spring [3]. Seroepidemiology studies in the United States [4] and Italy [5] indicated that over 90% of children had antibodies for HBoV by 4 years of age.

PCR amplification of viral nucleic acids is the most commonly used technique for the detection of HBoVI in respiratory samples. However, the true clinical impact of a positive PCR result is often difficult to assess as HBoVI DNA may persist for months in the respiratory tract. The DNA can be encountered in both symptomatic patients and asymptomatic individuals, as well as a cofinding in respiratory infections caused by other pathogens [6].

Nevertheless, causative associations between HBoVI infection and severe illness, including unexplained severe lower RTI [7] and encephalitis [8], have been described. This emphasizes the need for novel diagnostic methods for accurate and rapid identification of clinically relevant HBoVI infections. Several techniques for the diagnosis of HBoVI infection have been developed. These include serology [9], mRNA reverse transcriptase (RT) PCR [10] and DNA PCR in serum and in nasal samples [11]. However, these methods are cost and labour intensive and are only available in highly specialized diagnostic laboratories.

The mariPOC test system (ArcDia International Oy Ltd., Turku, Finland) provides a rapid alternative for HBoVI detection. mariPOC is an automated and point-of-care compatible test for rapid and simultaneous detection of antigens of eight respiratory viruses (influenza A and B, respiratory syncytial virus, adenovirus, human metapneumovirus and parainfluenza type 1, 2 and 3 viruses) and *Streptococcus pneumoniae* from a single nasopharyngeal sample. Detection of antigens is based on separation-free two-photon excitation fluorometry [12]. Recently a method to detect HBoVI antigen was added to the test panel. The new HBoVI antigen test has an analytical sensitivity of 3 ng/mL for recombinant HBoV-like particles

(VP2), and it has shown good correlation with HBoVI mRNA PCR designed to detect acute infection cases only (Toivola *et al.*, poster presented at 25th European Congress of Clinical Microbiology and Infectious Diseases, 2015).

Here we describe the use of the HBoVI antigen test for the detection and monitoring of HBoVI in nasopharyngeal samples.

Case Report

A previously healthy girl, 5 months of age, developed symptoms of rhinorrhoea, cough and fever (temperature up to 39°C). The consulting paediatrician diagnosed an upper RTI with no lower respiratory tract involvement or signs of otitis. After written parental consent was obtained, a nasopharyngeal swab was taken on day 2 of illness and tested by the mariPOC for virologic diagnosis. Within 20 minutes the sample tested positive for HBoVI antigen. Retrospective PCR testing confirmed the diagnosis: the nasopharyngeal sample tested positive for HBoVI DNA by quantitative PCR [13] with a load of 7.7×10^7 copies/mL and for HBoVI mRNA by RT-PCR [10] with a Ct value of 26. To follow the course of infection, nasopharyngeal samples were taken on days 3, 4 and 5 from the onset of symptoms. While rhinorrhoea and mild cough continued, the fever declined on day 3. An initial increase in mariPOC assay signal was followed by a gradual decrease (Fig. 1). The viral antigen concentrations were extrapolated from a dose–response curve of a positive control dilution series. On day 6, the RTI symptoms were mild, which coincided with a decreasing HBoVI antigen load.

Discussion

With the mariPOC rapid test system, we were able to *in vitro* diagnose and monitor for the first time the course of a primary HBoVI RTI by directly targeting the virus proteins. Compared to standard laboratory diagnostics, this rapid test is easy to perform and may allow for a clinically accurate diagnosis of HBoVI infection. The initial HBoVI antigen detection was confirmed by both DNA and mRNA PCR. The costs of the mariPOC multianalyte tests are comparable with other antigen detection assays, such as direct fluorescent antibody testing. Compared to rapid and easy to perform nucleic acid detection methods, e.g. PCR assays with integrated sample preparation, mariPOC immunoassay is a less expensive alternative.

mariPOC test results are typically reported as qualitative. In this study we followed the course of the HBoVI infection by using the quantitative property of the underlying two-photon

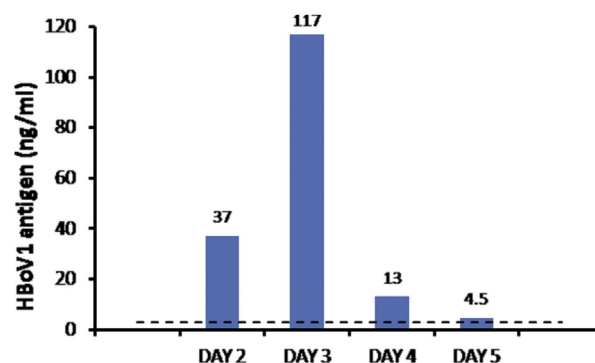


FIG. 1. Detection of human bocavirus 1 (HBoVI) antigen by mariPOC test (ArcDia International Oy Ltd., Turku, Finland) during course of respiratory infection. Dashed line indicates cutoff for positive finding. For each day of infection, nasopharyngeal sample was analysed by HBoVI antigen test.

excitation technology. However, the nature of swab sampling limits the quantitativeness of the data to semiquantitative. We showed the duration of this HBoVI active infection to be approximately 1 week. The patient was HBoVI antigen positive the morning after onset of symptoms, and HBoVI antigen positivity lasted until day 5, when the signal barely exceeded the diagnostic threshold. No sample was available beyond day 5, while the decline in antigen since day 3 and the near-cutoff result on day 5 strongly suggest that the patient would have been negative on day 6. These results are in line with the time span of other respiratory viruses [14] and demonstrate once more that rapid testing should be done as soon as possible after the onset of symptoms and at the latest within 5 to 6 days.

While most HBoVI infections are self-limited, they sometimes lead to life-threatening conditions. The availability of a rapid and accurate test for HBoVI is likely to be useful in differential diagnoses and development of therapy, and will increase our understanding of the epidemiology and clinical impact of HBoVI infections.

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Conflict of Interest

HT, HA and JOK are R&D employees at ArcDia International Oy Ltd. The other authors declare no conflict of interest.

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